

## Determination of Nicotine and its Metabolites in Urine by HPLC after DETBA Derivatization

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A method for the simultaneous determination of nicotine and 9 of its metabolites, i.e., cotinine, 3'-hydroxycotinine, 5'-hydroxycotinine, nicotine-N'-oxide, norcotinine, normicotine, 4-(3-pyridyl)-4-hydroxybutyric acid, 4-(3-pyridyl)-4-oxybutyric acid, and 3-pyridylacetic acid, in human urine is described. The phase-2 metabolites nicotine-N-glucuronide, cotinine-N-glucuronide, and 3'-hydroxy-O-glucuronide are determined in a 2<sup>nd</sup> chromatographic run after treatment of the urine sample with  $\beta$ -glucuronidase and calculation of the difference between the free and total analytes. Approximately 95% of the known metabolites excreted in human urine can be determined.

The method is based on our method for HPLC analysis after DETBA derivatization [Rustemeier et al., *J. Chromatogr.* 613, 95-103, (1993)]. Modification of derivatization conditions, solvent composition, pH, flow, and detection wavelengths has resulted in significant improvements in the method. Derivatization and chromatography are fully automated and the chromatography is performed within 13 min.

The method was used for biomonitoring of the nicotine uptake and the determining of the relative distribution pattern of nicotine and its metabolites in urine of smokers.

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